High Plant Growth Temperatures Increase Antioxidant Capacities in Strawberry Fruit

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Abstract

The influence of four day/night growing temperature combinations (18/12, 25/12, 25/22, 30/22 ^{o}C) on phenolic acid, flavonol, and anthocyanin content and their antioxidant activities against peroxyl radicals (ROO $^{\cdot}$), superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$), and singlet oxygen ($^1O_2$,) in fruit juice of 'Earliglow' and 'Kent' strawberry (*Fragaria* x *ananassa* Duch.) were studied. The content of cyanidin- based anthocyanins, were much lower than pelargonidin-based anthocyanins. High day/night temperature conditions significantly enhanced *p*-coumaroylglucose, dihydroflavonol, quercetin 3-glucoside, quercetin 3-glucuronide, kaempferol 3-glucoside, kaempferol 3-glucuronide, cyanidin 3-glucoside, pelargonidin 3-glucoside, pelargonidin 3-rutinoside, cyanidin 3-glucoside-succinate and pelargonidin 3-glucoside-succinate content in strawberry juice. Plants grown at low day and night temperatures (18/12 ^{o}C) generally had the lowest anthocyanin contents. Plants grown at the highest day/night temperatures (30/22 ^{o}C) produced fruit with the most phenolic content as well as antioxidant capacity. Fruit of 'Kent' strawberry had higher contents of phenolic acid, flavonols, anthocyanins and antioxidant capacities compared to fruit of 'Earliglow' strawberry under all temperature regimes.

INTRODUCTION

Fruits and vegetables contain high levels of antioxidant compounds, which provide protection against harmful free radicals and have been associated with lower incidence and mortality rates of cancer and heart disease in addition to a number of other health benefits (Ames et al., 1993; Velioglu et al., 1998). Our previous studies have shown that thornless blackberries (*Rubus* sp.), blueberries (*Vaccinium* spp.), cranberries (*Vaccinium macrocarpon* Aiton), raspberries (*Rubus idaeus* L. and *Rubus occidentalis* L.) and strawberries (*Fragaria* x *ananassa* Duch.) have high antioxidant capacities (Wang and Jiao, 2000; Wang and Lin, 2000). However, no information is available on the effect of environmental factors such as growing temperatures on scavenging capacity of strawberry against active oxygen species. The present study evaluated the effects of four day/night growing temperatures (18/12, 25/12, 25/22 and 30/22 ^{o}C) on antioxidant activities against ROO $^{\cdot}$, $O_2^{\bullet-}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$ radicals associated with changes in anthocyanins and other phenolic compounds of strawberry.

MATERIALS AND METHODS

Plant Materials and Experimental Plans : Uniform sized, approximately one-year-old plants of Earliglow and Kent cultivars were used. The plants were propagated by runner-tip cuttings in June and plants were grown in 2- liter plastic pots containing Pro Mix BX (Premier Brands Inc., Stamford, Conn.) in a greenhouse. Radiation sources in the greenhouse consisted of natural daylight and Watt-Miser incandescent lamps (Nela Park, Cleveland, OH) that provided a PAR around 700 μmol $m^{-2}s^{-1}$ for 14h/d (0600-2000h). Temperatures were set at around 25°C during the day and 20°C at night. During the growing season, all plants were watered daily and fertilized biweekly with 150 ml/ plant of Peters fertilizer (20-20-20, N/P/K). Prior to initiation of temperature treatments, plants were exposed to ambient winter temperatures in Beltsville, Maryland, USA, in an

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unheated greenhouse from October to February. Plants were then moved to a heated greenhouse (25°C during the day and 20°C at night) for approximately 1.5 months to force flowering. Blossoms were self-pollinated by hand using a small brush. Plants with the most fruit (at least ten fruits per plant) at their green fruit stage were selected for the growth chamber experiments. Forty plants each of 'Earliglow' and 'Kent' were removed from the greenhouse in March and divided into lots of 10 plants. One lot of each cultivar was randomly placed in four growth chambers set at day/night temperatures of 18/12, 25/12, 25/22 and 30/22°C. The plants were in growth chambers for 1.5 months. The photoperiod for each growth chamber was 14 hr (6:00-20:00 hr) with a PAR around 700 μmol $m^{-2}s^{-1}$ at plant height. Firm red-ripe fruits free from defects or decay were harvested from each cultivar in each growth chamber during fruit ripening and the berries were cut into small slices, mixed, and then used for analyses.

Fruit Sample Preparation : To prepare the juice samples, three-100g samples of berries from three replicates of each cultivar of each treatment were pulverized and then centrifuged at 14,000 *g* for 20 minutes at 4°C. The supernatants were transferred to vials, stored at -80°C and then used for analyses.

Peroxyl Radicals (ROO ·) ORAC Assay : The procedures for the ORAC assay on strawberries were modified from a previously described method by Cao et al. (1993). The ORAC value refers to the net protection area under the quenching curve of R-PE in the presence of an antioxidant. The final results (ORAC value) were calculated and expressed using Trolox equivalents per gram on a fresh weight basis.

Superoxide Radical ($O_2^{\bullet-}$) Assay : The assay for superoxide radical ($O_2^{\bullet-}$) was determined using the methods of Elstner and Heupel (1976). The final results were expressed as percent inhibition of $O_2^{\bullet-}$ production in the presence of fruit juice. The scavenging capacity of α-tocopherol at various concentrations (1 to10 μg) on superoxide radical ($O_2^{\bullet-}$) was measured and used for determining the $O_2^{\bullet-}$ scavenging capacity of fruit juice. The antioxidant capacity of fruit juice against the $O_2^{\bullet-}$ value was expressed as μmole of α-tocopherol equivalent per gram fresh weight.

Hydroxyl Radical (OH ·) Assay : The assay for hydroxyl radical (OH·) was determined using the methods of Richmond et al. (1981). Relative scavenging efficiency (% inhibition of hydroxylation) of fruit juice was estimated from the difference in absorbance (OD) with and without addition of the fruit juice. The scavenging capacity of chlorogenic acid at various concentrations (1 to10 μg) on hydroxyl radical (OH·) was measured and used for determining the OH· scavenging capacity of fruit juice. The antioxidant capacity of fruit juice against OH· value was expressed as μmole of chlorogenic acid equivalent per gram fresh weight.

Hydrogen Peroxide (H_2O_2) Assay : The assay for hydrogen peroxide in fruit juices of blackberry, raspberry, cranberry, blueberry and strawberry was carried out following procedures previously described by Patterson et al. (1984). The scavenging capacity of ascorbate at various concentrations (1 to10 μg) on hydrogen peroxide (H_2O_2) was measured and used for determining the H_2O_2 scavenging capacity of fruit juice. The antioxidant capacity of fruit juice against H_2O_2 value was expressed as μmole of ascorbate equivalent per gram fresh weight.

Singlet Oxygen ($^1O_2$) Assay : The production of singlet oxygen ($^1O_2$) by sodium hypochloride and H_2O_2 was determined by using a spectrophotometric method according to Chakraborty and Tripathy (1992). Relative scavenging efficiency (% inhibition production of $^1O_2$) of fruit juice was estimated from the difference in absorbance of *N, N*, dimethyl-*p*- nitrosoaniline with and without the addition of fruit juice. The scavenging capacity of β-carotene at various concentrations (1 to10 μg) on singlet oxygen ($^1O_2$) was

measured and used for determining the $^1O_2$ scavenging capacity of fruit juice. The antioxidant capacity of fruit juice against $^1O_2$ value was expressed as μmole of β-carotene equivalent per gram fresh weight.

HPLC Analysis of Strawberry Anthocyanins and Phenolic Compounds : Fruit samples of 5 g were extracted twice with 15 mL of acetone. Extracts were concentrated to 1 mL. The concentrated sample was dissolved in 10 mL acidified water (3% formic acid) and then passed through a C_{18} Sep-Pak cartridge (Waters). Anthocyanins and other phenolics were adsorbed onto the column, then recovered with 2.0 mL of acidified methanol containing 3% formic acid. The methanolic extract was passed through a 0.45-μm membrane filter (Millipore, MSI, Westboro, MA) and 20 μL was analyzed by HPLC (Waters Corporation, Milford, MA). HPLC system equipped with two pumps (600 E) coupled with a photodiode array detector (Waters 990 Series). Samples were injected onto a reverse phase NOVA-PAK C_{18} column (150×3.9 mm, particle size 4 μm) with a guard column (NOVA-PAK C_{18}, 20x3.9 mm, particle size 4 μm). The mobile phase was acidified water containing 2.5% formic acid (A) and acetonitrile (B) in a linear gradient from 5% to 20% B in the first 15 min, followed by a linear gradient from 20 to 30% B for 5 min, then an isocratic mixture for 5 min, followed by a linear gradient from 30 to 90% B for 5 min, an isocratic mixture for 2 min before returning to the initial conditions. The flow rate was 1.0 mL/min and the wavelengths of detection were set at 320, 350, and 510 nm. Data were collected by the Waters 990 3-D chromatography data system. Retention times and spectra were compared to pure standards.

RESULTS

Growth temperatures during day and night significantly affected antioxidant activity in the juice of two strawberry cultivars (Earliglow and Kent) (Fig.1). Strawberry growth under high temperature conditions significantly enhanced fruit $ROO^{\cdot}$ absorbance capacity, as well as $O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$ and $^1O_2$. Fruit produced from plants grown in cool day and night temperatures (18/12 ^oC) generally had the lowest antioxidant capacity. Fruit of 'Kent' had higher values of antioxidant capacity compared to 'Earliglow' fruit. In both cultivars, an increase in night temperature from 12 to 22 ^oC, with the day temperature kept constant at 25 ^oC, resulted in a significant increase in values of fruit antioxidant capacity. The highest day/night temperatures (30/22 ^oC) yielded fruit with the most $ROO^{\cdot}$ absorbance capacity, as well as $O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$ and $^1O_2$ absorbance capacity (Fig.1).

High growth temperatures (25 and 30 ^oC) significantly enhanced the content of phenolic acids and flavonoids such as *p*-coumaroylglucose, cyanidin 3-glucoside, pelargonidin 3-glucoside, cyanidin 3-glucoside-succinate and pelargonidin 3-glucoside-succinate (Fig. 2). Fruit from plants grown in the cool day and night temperature (18/12 ^oC) treatment generally had the lowest phenolic acid, flavonols, and anthocyanins. An increase in night temperature from 12 to 22 ^oC, with the day temperature kept constant at 25 ^oC, resulted in a significant increase in the content of flavonoids in the fruit of 'Earliglow' and 'Kent'. The highest day/night temperature (25 and 30 ^oC) yielded fruit with highest amount of phenolic acid, flavonols, and anthocyanins. 'Kent' strawberry had higher values of these components compared to 'Earliglow' (Fig. 2).

DISCUSSION

Growth temperatures affected strawberry fruit antioxidant capacity. When the day/nighttemperature became warmer, the fruit surface and flesh colors became redder and darker and their antioxidant content significantly increased. Fruit from the plants grown in day/night temperature 30/22 ^oC had the highest percent inhibition of the free radicals $O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$ and $^1O_2$, while fruit grown at 18/12 ^oC had the lowest capacity for each of these activities.

The most important single group of phenolics in strawberries are flavonoids which consist mainly of catechins, proanthocyanidins, anthocyanidins and flavones, flavonols and their glycosides. These flavonols are effective antioxidants (Rice-Evans et al., 1995;

Rice-Evans and Miller, 1996). Kaempferol and quercetin are potent quenchers of $ROO^{\cdot}$, O_2 and $^1O_2$ (Larson, 1988). Quercetin and other polyphenols have been shown to play a protective role in carcinogenesis by reducing bioavailability of carcinogens and kaempferol has low antioxidant capacity against peroxyl radicals. The antioxidant capacities for quercetin and kaempferol are 3.29 and 2.67, respectively (Cao et al., 1997). Higher growth temperatures significantly enhanced flavonol content in strawberry fruit and juices, and fruits having high flavonol contents also had high antioxidant activities.

Two anthocyanidin glycosides, pelargonidin 3-glucoside and cyanidin 3-glucoside, are almost exclusively responsible for the red color of strawberries. It has been shown that anthocyanidins are strong antioxidants with free radical scavenging properties attributed to the phenolic hydroxyl groups attached to ring structures (Rice-Evans et al., 1995; Rice-Evans and Miller, 1996; Wang et al., 1997). The hydroxyl radical scavenging activities of flavonoids increase with the number of hydroxyl groups substituted on the B-ring, especially at C-3' (Rice-Evans et al., 1995; Wang et al., 1997). All flavonoids such as cyanidin, with 3', 4'-dihydroxy substitution in the B ring and conjugation between the A- and B-rings, possess antioxidant activity (Dziedzic and Hudson et al., 1983) and have antioxidant potentials four times that of Trolox (Rice-Evans et al., 1995). Epidemiologic studies have shown a correlation between an increased consumption of antioxidants and a reduced risk of cardiovascular disease and certain types of cancer (Rice-Evans and Miller, 1996; Cao et al., 1997; Wang et al., 1997).

In summary, strawberry fruit juice contains flavonoids with potent antioxidant properties. Different hydroxylation and glycosylation may modulate their antioxidative properties. Growth temperatures affect their phenolic acid, flavonol, and anthocyanin content and antioxidant capacity. The concentration of flavonoids in strawberry juice was related to antioxidant activity against $ROO^{\cdot}$, $O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$, and $^1O_2$ radicals. High temperature conditions significantly enhanced flavonoids contents and antioxidant capacities in fruit.

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Figures

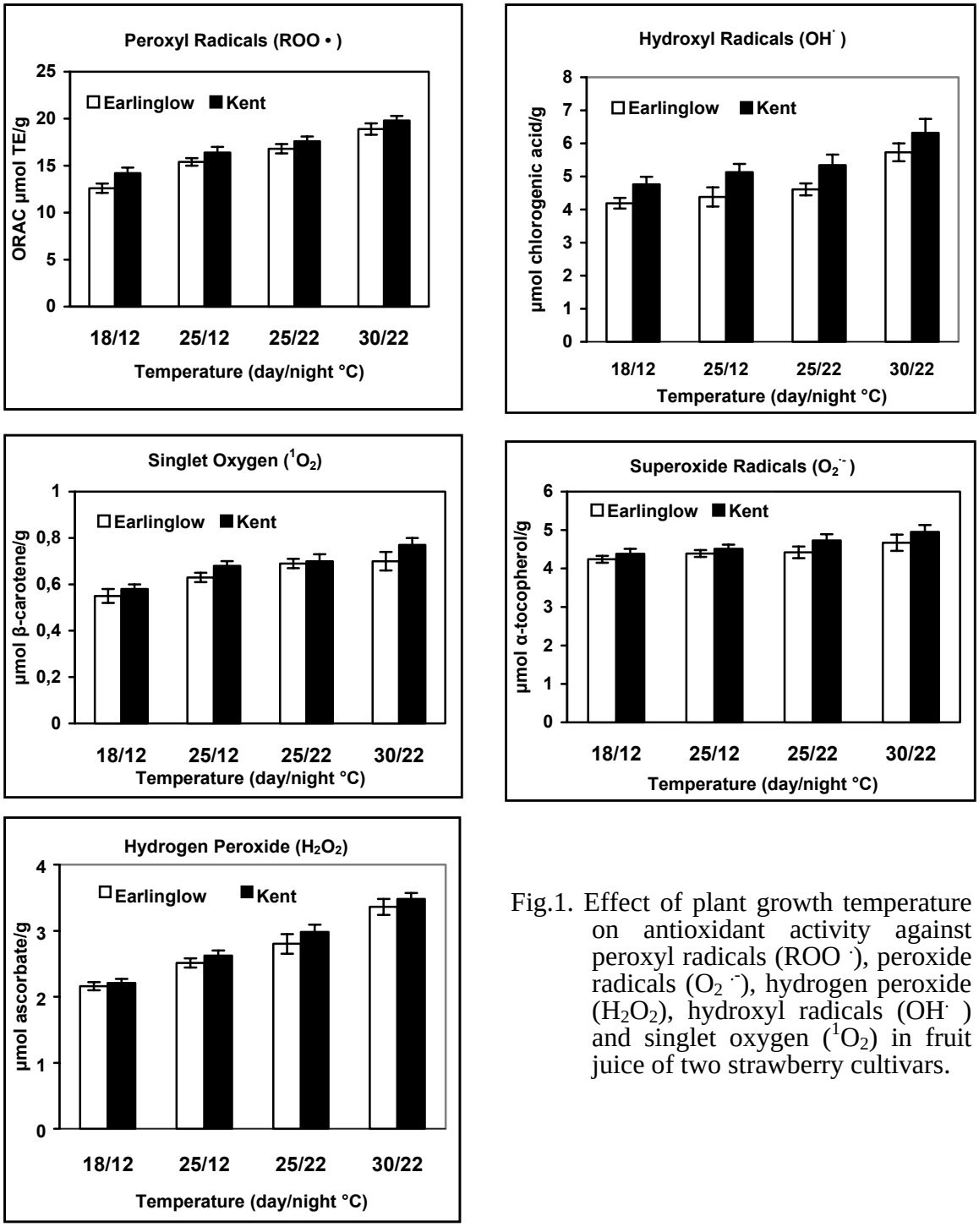

Fig.1. Effect of plant growth temperature on antioxidant activity against peroxyl radicals (ROO ·), peroxide radicals (O_2 ·⁻), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH·) and singlet oxygen ($^1O_2$) in fruit juice of two strawberry cultivars.

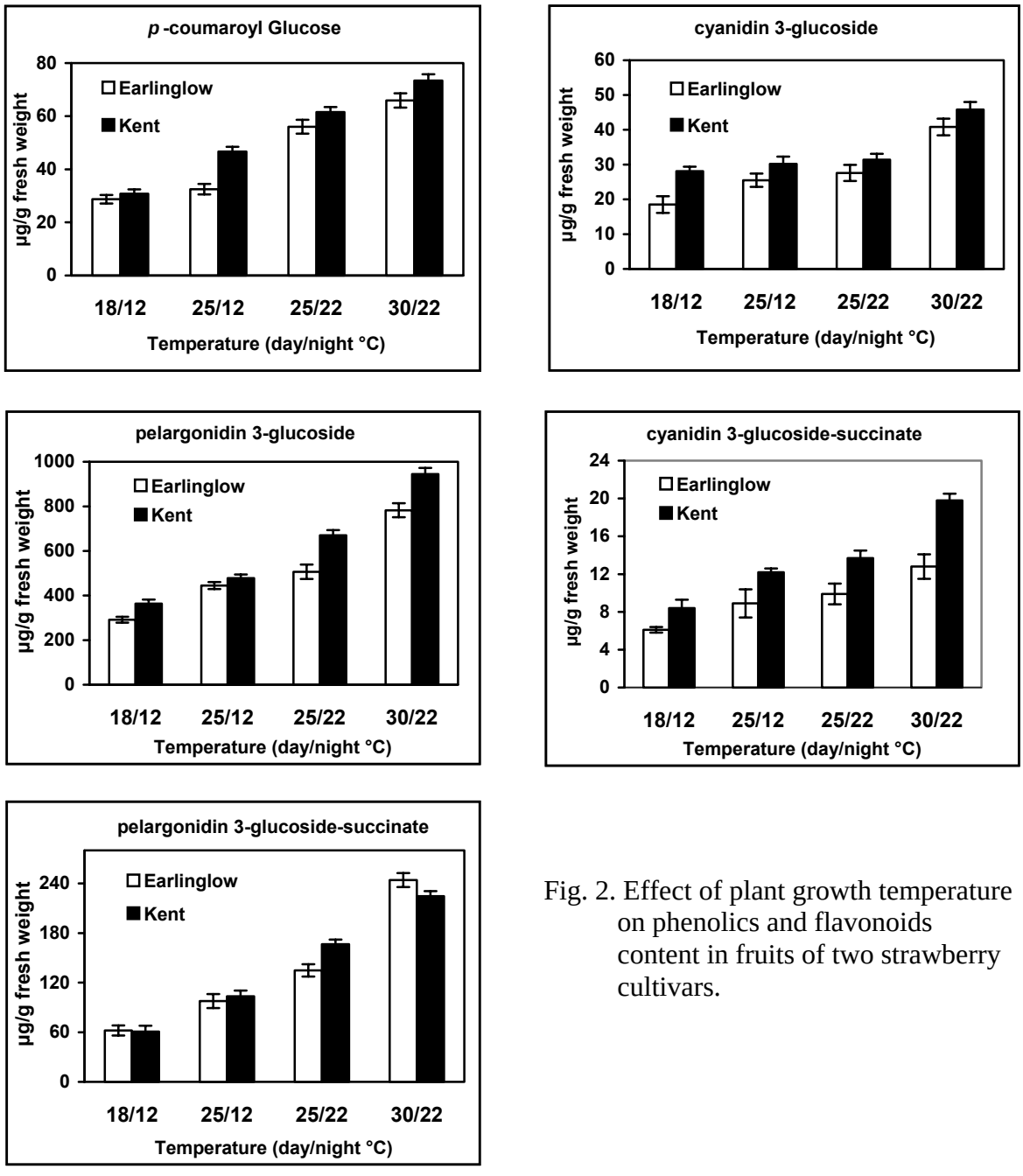

Fig. 2. Effect of plant growth temperature on phenolics and flavonoids content in fruits of two strawberry cultivars.